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A Simple Photometer for Precise Determination of Dissolved Oxygen Concentration by the Winkler Method with Recommendations for Improving Respiration Rate Measurements in Aquatic Organisms

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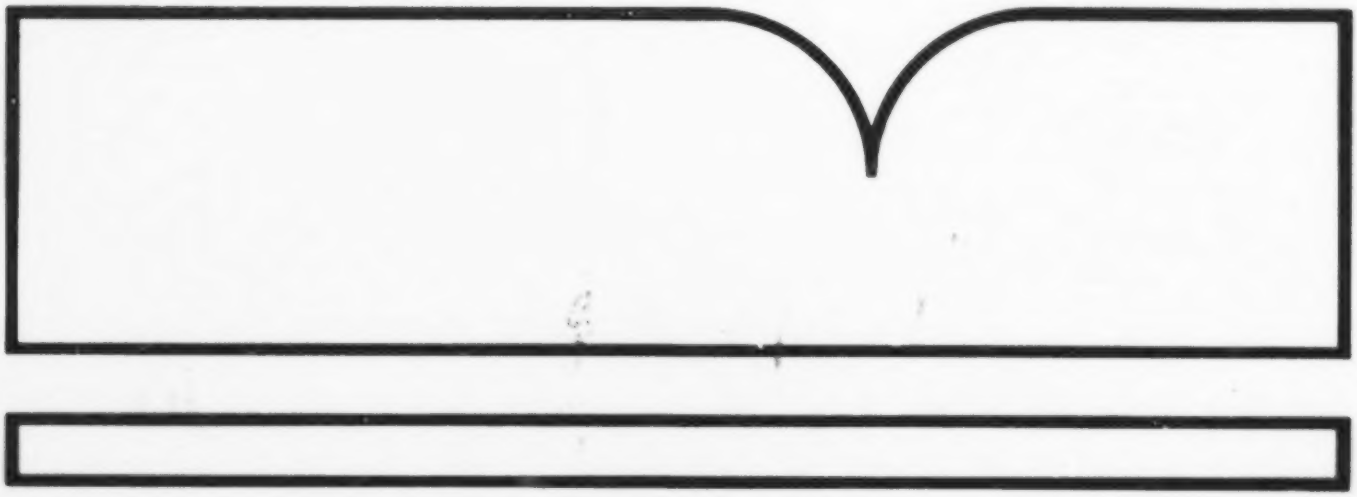

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16. ABSTRACT

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A Simple Photometer for Precise Determination of Dissolved Oxygen Concentration by the Winkler Method with Recommendations for Improving Respiration Rate Measurements in Aquatic Organisms

ABSTRACT: A simple inexpensive photometer designed for Winkler titration end-point detection is described. The precision of replicate dissolved oxygen measurements using this instrument was 0.06–0.22%. This high precision is needed to measure the small changes in dissolved oxygen concentration for determining the respiration rate of small aquatic organisms. Other recommendations for improving precision are also presented.

Introduction

The respiration rate of small aquatic organisms is usually measured by the difference in oxygen concentration between a control (no organisms) and experimental (with organisms) bottle after an incubation period. Various methods have been used for determining oxygen concentration, but the most commonly used are either oxygen electrodes or the Winkler titration. Oxygen electrodes offer the advantage of instantaneous and continuous monitoring of oxygen concentration but are less sensitive than the Winkler titration for detecting small changes. Most are readable to only 0.03 ppm (0.02 ml/l) and have a precision of ±0.5% (Hartwig and Michael 1978). The Winkler titration on the other hand is accurate to ±0.1% (±0.005 ml/l at 5.000 ml/l). In practice, however, its precision depends on the endpoint detection method (Carpenter 1965). Photometric endpoint detection is the most sensitive method, up to 100 times more sensitive than visual endpoint detection and almost 10 times more sensitive than the amperometric technique (Carpenter 1965). It is based on changes in absorbance during titration due to changes in the concentration of the triiodide complex. This complex is strongly absorbing in the ultraviolet at 350 nm. Measuring these absorbance changes during titration is somewhat of a problem in the usual laboratory spectrophotometer. Transfer of the iodine solution results in considerable error (Carritt and Carpenter 1966), making titration of the entire sample bottle desirable. Commercially available spectrophotometers generally do not have a sample compartment large enough to accommodate the sample bottle and stirrer. In addition, if these difficulties are overcome, introducing the thiosulfate requires drilling holes in the sample compartment allowing interference from stray light during other uses. Recently, Bryan, et al. (1976) and Hartwig and Michael (1978) have reported on photometric devices designed for Winkler endpoint detection. Unfortunately, the instrument described by Bryan, et al. (1976) is difficult to build as the parts required are unobtainable, at least in this country. We felt that the instrument described by Hartwig and Michael was unnecessarily complicated and bulky. The instrument described in this paper has a simple stabilized light source and detector system and is quite inexpensive to build. In addition, a recorder is not required for very high precision titrations.

In addition to improvement of endpoint detection several other factors such as sample volume, thiosulfate concentration, and precision of the titrant delivery system also alter the precision of the Winkler method. In addition, the magnitude of the difference in oxygen concentration between the control and experimental bottles directly affects the precision of the respiration rate measurement. We performed replicate oxygen concentration determinations altering these factors individually and assessing their effect on the precision of the Winkler method.

Methods

The photometer consists of a highly regulated supply for the tungsten lamp, a narrow band pass filter for wavelength selection, an ultraviolet sensitive photodiode detector, and three operational amplifiers to gain and condition the signal from the photodiode. Signal readout is accomplished via a digital panel meter. A schematic of the electronics is shown in Fig. 1.

The heart of the regulated lamp supply is the voltage regulator (VR). This is a Fairchild μA78M05 integrated circuit (IC). According to the manufacturer's literature, the minimum regulation stability is typically ±50 mV under small current load. This corresponds to a regulation stability of ±0.1%. The pass transistor Q_1 is a Fairchild 2N4399 pnp power transistor. This transistor, with proper heat dissipation is capable of a 15 Amp output. The lamp only requires 2–4 Amp to operate. This configuration not only supplies the necessary current to the lamp but also maintains a low current on the VR so that it can maintain maximum regulation stability. The power transistor was mounted on the back of the supply enclosure with a heat sink for adequate power dissipation. In operation, the lamp power supply has been found to be at least a factor of five more stable than the minimum stability guaranteed by the manufacturer.

The step-down transformer, a Stancor RT-204 (T in Fig. 1), has a multitap secondary so that several different output voltages are available for selection. This feature is important because the VR requires an input voltage at least two volts greater than its operating voltage to maintain proper regulation. However, if the applied voltage is too large, this excess power must be dissipated as heat in Q_1, which can result in premature failure of the power transistor if adequate heat sinking is not available.

By adjusting R_3, a 50 Ω one turn variable resistor, the voltage applied to lamp L can be varied from 5.0 to 6.3 volts DC. Generally, adequate lamp intensity and stability was available at the lower voltage settings.

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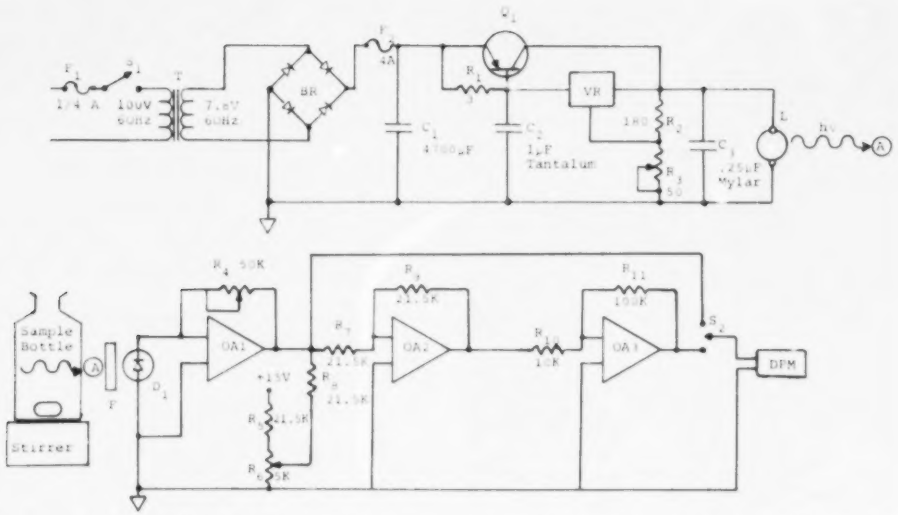

Fig. 1. Schematic of the electronics. The Bridge Rectifier (BR) is a Radio Shack Model 276-1180. Fuses F_1 and F_2 are standard 1¼ inch Buss fuses. Not shown are the power connections to the operational amplifiers and the amplifier power supply, a Teldyne Philbrick Model TP-2212.

Increasing lamp voltage to the maximum significantly shortens lamp life. The lamp employed is an American Optical Model 365 tungsten microscope lamp. The lamp housing has a built-in focusing lens so that the output is a circular beam approximately 2 cm in diameter at the detector. The lamp has a bayonet type mounting connector. If the lamp was not firmly seated in its mount, instability developed with time, probably due to corrosion build-up on the contacts.

The light beam was filtered with a narrow bandpass filter (F) centered at 350 nm (Kodak) and detected with a United Technology pin 10-UV photodiode (D_1). The current output from D_1 was converted to a voltage by operational amplifier OA1. All operational amplifiers employed were Teledyne Philbrick Model 1421 FET amplifiers. The detector gain could be varied by adjusting R_1, a 10 turn 50K variable resistor. This voltage could be offset to zero by adjusting R_6, a 10 turn 5K variable resistor. Amplifier OA2 functions as a summing amplifier to add the output from OA1 and the offset voltage. Resistors R_5, R_7, R_8, and R_9 in the summing network are all 21.5K metal film, 1% precision resistors. This particular resistance value was chosen only because the resistors were readily available in our laboratory. The resulting voltage from OA2 is then gained ×10 by OA3. The signal was then detected by a Simpson Model 2850 3½ digit digital panel meter (DPM). Either the output of OA1 could be read directly on the DPM or the gained and offset result could be ready by changing position on switch S_2. The detector system has short term (less than one minute) stability of ±1 mV in the absence of a sample bottle. Some long term drift (greater than one hour) was observed and is probably due to the lamp aging. However, this drift had no effect on individual titrations. The instrument is shown in Fig. 2. The photometer is extremely simple, both in design and use and has proven to be very reliable during several months of use. The instrument is also very inexpensive to construct, costing less than $300.00 (1977 prices).

A one volt recorder readout can be obtained by simply hooking into switch S_2. In addition, several pins are available on a back panel socket on the DPM where a digital signal is brought out, and it should be fairly easy to interface the spectrophotometer to a laboratory minicomputer. Neither of these features have as yet been utilized on our instrument.

Replicate samples were prepared for each test from a 20 L carboy of filtered sea water at room temperature which was stirred for 1 hr prior to sampling. Samples were fixed immediately using the reagents and procedure recommended by Carritt and Carpenter (1966). However, we used 0.5 ml of each reagent rather than 1.0 ml as our sample volume (60 ml) was approximately one-half of theirs (125 ml). The entire sample bottle was then titrated to endpoint with 0.04 N (10 g $Na_2S_2O_3 \cdot 5H_2O$/l) thiosulfate. Endpoints for all the samples were determined photometrically except those done visually for comparison.

To prepare for a series of titrations, the instrument was warmed up for about 30 min. The millivolt output shown by the panel meter was adjusted to approximately 1.500 V using the detector gain without a sample bottle in the light path. The presence of a sample bottle further focuses the light beam and increases the millivolt reading. We aligned the light beam with the detector by adjusting the detector position until the millivolt output was at maximum with a sample bottle in the light path. The focus point of the ultraviolet appeared to be somewhat different from that of the visible light. The instrument was then switched to the read setting and the millivolt output adjusted to zero. This

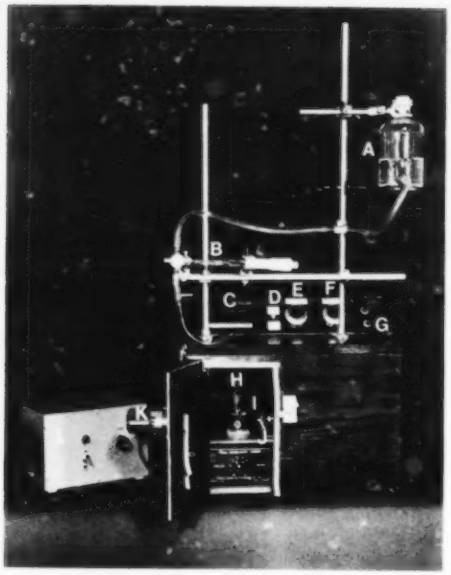

Fig. 2. The spectrophotometer ready for titration. The main unit measures 40 cm w × 36 cm h × 34 cm d. A. Thiosulfate solution, B. Three-way stopcock and microburet, C. Panel readout, D. Function switch, E. Zero adjustment, F. Detector gain, G. On-off switch, H. Sample bottle, I. Sharp-cut filter, J. Detector, K. Lamp, L. Lamp power supply.

allowed us to adjust for changes in absorbance due to bottle differences and still keep the total millivolt change during titration relatively constant. With the stirrer on, thiosulfate was then added until a constant millivolt output was reached. Generally, the increase in millivolt readings, due to disappearance of the ultraviolet absorbing triiodide complex, took place over the last 0.050–0.060 ml of thiosulfate addition. The change in millivolt output was about 25 mV/ul thiosulfate. Noise in individual millivolt readings due to stirring was ±3 mV. Therefore, detection limits of the instrument are less than the precision of the thiosulfate

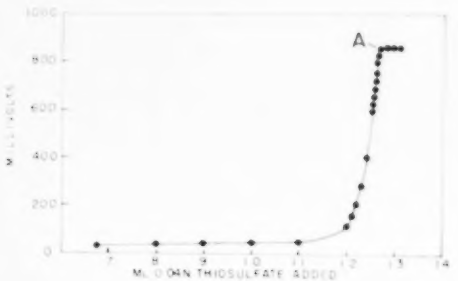

Fig. 3. The exponential curve resulting when the iodine solution is titrated to endpoint with thiosulfate. The curve is effectively linear (r^2 = 0.998) over the last 60 μl thiosulfate added. A. Endpoint.

delivery system in that we should be able to detect additions of 0.2 μl thiosulfate by a change of about 5 mV.

Results and Discussion

Photometric versus Visual Endpoint Detection

The change in millivolt reading over the course of the titration (Fig. 3) is exponential and approaches linearity over the last 50 μl of thiosulfate addition with r^2 = 0.988. To insure against overshooting the endpoint several millivolt readings can be taken during the course of the titration and an endpoint calculated by substituting the maximum reading in the calculated regression equation.

We prepared a series of 32 replicate 60 ml BOD bottles at room temperature to test the precision of the oxygen concentration determinations using our instrument. All samples were drawn from a continuously flowing siphon from a 20 l carboy of sea water which was stirred for 1 hr prior to sampling. This procedure allowed us to calculate the precision of four replicates as well as detect any systematic decrease in precision with time, such as may be found in visual endpoint detection due to operator fatigue. Each titration took 3.5–4.0 min, and the hourly average was 15–16 titrations. Our precision compares favorably with that shown by Hartwig and Michael (1978) and ranges from

TABLE 1. Changes in precision of oxygen concentration measurements due to various factors.

Endpoint Detection				Sample volume				Thiosulfate Concentration			
Visual		Photometric		125 ml		60 ml		0.04 N		0.14 N	
CV (%)*	n	CV (%)	n	CV (%)	n	CV (%)	n	CV (%)	n	CV (%)	n
0.44	n	0.11	4	0.08	4	0.16	4	0.07	4	0.94	4
1.26	4	0.11	4	0.14	4	0.21	4	0.08	4	0.74	7
0.25	4	0.13	4								
1.74	4	0.15	4								
0.31	4	0.19	4								
1.90	4	0.22	4								
0.16	4	0.06	4								
0.24	4	0.07	4								

* CV (%) = $(S_x/\bar{x}) \times 100$.

0.06–0.22% (Table 1), despite the decrease in sample volume which in itself halves the precision as discused below. Using the same sampling procedure, sample volume, thiosulfate concentration, and titrant delivery system we titrated a similar series using visual endpoint detection. This procedure resulted in a coefficient of variation of 0.16–1.90% (Table 1). The photometric endpoint detection method, therefore, gives about three to ten times greater precision. In addition, the range of values of the coefficient of variation for replicate groups was less, possibly due to the lessened effects of operator fatigue and changes in ambient light.

Sample Volume

Replicate samples were drawn and preserved in 60 ml BOD bottles and 125 ml glass stoppered flasks to compare the effect of sample volume on precision. The endpoint was determined photometrically in both instances. The coefficient of variation for the 60 ml samples was 0.16–0.21% (n = 4) and 0.08–0.14% for the 125 ml samples, approximately half that of the 60 ml samples or twice as precise. However, increasing sample volume must be weighed against the increased time spent sorting additional animals to produce the same change in oxygen concentration in a larger volume of water over the same incubation period.

Thiosulfate Concentration

A thiosulfate concentration of 0.14 N (35 g $Na_2S_2O_3 \cdot 5H_2O$/l) is recommended to give a sharp endpoint when using starch indicator and visual endpoint detection (Carritt and Carpenter 1966). We routinely used a 0.04 N thiosulfate solution. A lower normality was used as our sample volume was only 60 ml and refilling the buret during a titration was unnecessary even at this lower concentration. With experience visual endpoint detection was reasonably precise (Table 1) although the color change at endpoint was not as clearcut as with more concentrated thiosulfate. Photometric endpoint detection eliminates the drawback of a less clearcut change. Replicate 60 ml samples titrated in this manner using the two different thiosulfate concentrations give a coefficient of variation of 0.74–0.94% (n = 4) for 0.14 N thiosulfate and 0.07–0.14% (n = 4) for 0.04 N thiosulfate (Table 1). Thus a reduction in thiosulfate normality significantly increased precision. However, decreasing the concentration of the thiosulfate to the point where more than one filling of the buret is required to titrate a sample should be avoided.

Titrant Delivery System

The thiosulfate solution used in the Winkler titration is usually introduced using a micrometer buret (Carritt and Carpenter 1966). When the precision of the endpoint detection is increased, such as with a photometric endpint detection method, the precision of the microburet may become limiting. The precision of a Gilmont Microburet (2.0 ml) and a Manostat Digipet (10 ml) were determineo by weighing a replicate series of volumes dispensed from each. The precision of the Gilmont Microburet was 0.15% (n = 3) or ±3.0 µl for 2.000 ml dispensed and that of the Manostat Digipet 0.19% or ±3.8 µl. Therefore, both burets had a comparable precision, but we noted that sometimes when we were weighing samples, the Manostat Digipet would not always register the total volume dispensed, with no indication in its mechanical operation that this was happening.

The spectrophotometric endpoint detection method can detect additions of 0.2 µl 0.4 N thiosulfate, therefore, both these microburets would be a limiting factor when attempting to increase precision. An ultraprecise 2.5 ml micrometer buret is now being marketed by Gilmont which may remove this limitation.

Magnitude of Oxygen Change

The magnitude of the oxygen difference between the control and experimental bottles directly affects the precision of the respiration rate measurement. Calculation of a series of hypothetical examples shows that for a 1% difference in oxygen concentration the coefficient of variation of the rate determination is one hundred and fifty times that of the concentration determinations. For a 10% difference it is fifteen times. Therefore, to detect subtle changes in respiration, highly precise concentration determinations are required or the difference in oxygen concentration between the control and experimental bottles must be large. To increase this difference the animal to volume ratio must be increased, which can cause changes in respiration due to crowding, or the incubation period increased, which can cause problems due to bacterial growth. In addition, decreases in oxygen concentration too far below saturation can affect respiration rate. Ikeda (1977) reports that 20% below saturation is the maximum change before the respiration rate of *Acartia tonsa* is affected.

In summary, for maximum precision in measuring the respiration rate or small aquatic organisms the following suggestions should be taken into account.

1. Photometric endpoint detection is more precise and consistent than visual endpoint detection.
2. Sample volume should be as large as possible.
3. Thiosulfate concentration should be kept as low as possible without requiring refilling the buret during a titration.
4. The precision of the titrant delivery system should be as high as possible.
5. The difference in the oxygen concentration between control and experimental bottles should be as large as possible but not exceed 20%.

Acknowledgments

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Literature Cited

Bryan, J. R., J. P. Riley, and P. J. Le B. Williams. 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. *J. Exp. Mar. Biol. Ecol.* 21:191–197.

Carpenter, J. H. 1965. The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* 10:135–140.

Carritt, D. E., and J. H. Carpenter. 1966. Comparison and evaluation of currently employed modifications of the Winkler method for determining dis-

solved oxygen in sea water; a NASCO report. *J. Mar. Res.* 24:286–318.

HARTWIG, E. O., AND J. A. MICHAEL. 1978. A sensitive photoelectric Winkler titrator for respiration measurements. *Environ. Sci. Technol.* 12:712–715.

IKEDA, T. 1977. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. *Bull. Plankton Soc. Japan* 24:19–28.

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